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Published in:
Cancer Cell

DOI:
10.1016/j.ccell.2017.01.010

Publication date:
2017

An oncogenic role for the ubiquitin ligase UBE2O by targeting AMPK-α2 for degradation

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There has been controversy regarding the role of AMPK in cancer, some of which may be due to functional differences between isoforms. In this issue of Cancer Cell, Vila et al report that UBE2O, a ubiquitin ligase over-expressed in some human cancers, specifically triggers the ubiquitination and degradation of AMPK-α2.

AMP-activated protein kinase (AMPK) is a cellular energy sensor involved in matching the supply of ATP from catabolism to the demand created by anabolism and other ATP-consuming activities, including cell division (Ross et al., 2016). As soon as demand out-strips supply the ADP:ATP ratio will increase, and this is amplified by the adenylate kinase reaction (2ADP ↔ ATP + AMP) into even larger increases in AMP:ATP ratios. Increases in ADP and AMP switch on AMPK by three complementary mechanisms, all antagonized by ATP: binding of AMP or ADP (i) promotes phosphorylation of Thr172 in the activation loop of AMPK by the upstream kinase, and (ii) inhibits Thr172 dephosphorylation, thus activating AMPK; while (iii) binding of AMP causes further allosteric activation of the phosphorylated kinase. AMPK then attempts to restore cellular energy homeostasis by switching on alternate catabolic pathways that generate ATP (especially the more energy-efficient oxidative pathways, rather than the glucose-hungry glycolysis), while switching off most ATP-consuming processes. The latter include protein synthesis, switched off indirectly by inactivation of mTOR complex-1 (Gwinn et al., 2008), and progress through the cell cycle, which is arrested in G1 phase (Fogarty et al., 2016).

The principal kinase phosphorylating Thr172, which is required for AMPK activation during energy stress although not directly activated by it, is LKB1. LKB1 was originally identified as a tumor suppressor mutated in an inherited susceptibility to cancer, Peutz-Jeghers syndrome, and was subsequently shown to be mutated in many sporadic cancers, especially lung adenocarcinomas. Since AMPK inhibits cell growth and proliferation, at first it seemed obvious that AMPK exerted the tumour suppressor effects of LKB1. However, this simple picture has recently become much more confused. The catalytic subunit of AMPK exists as two isoforms, α1 and α2 (Ross et al., 2016). While α2 knockout mouse embryo fibroblasts (MEFs) are sensitive to transformation by mutant H-Ras in vitro, and H-Ras-transformed α2-KO cells grow even more rapidly than wild type controls as tumors in vivo (correlating with reduced expression of p53), α1-KO MEFs are resistant to transformation in vitro and grow very poorly in vivo (Phoenix et al., 2012). In addition, in a synthetic lethal screen that searched for genes required for growth of cells over-expressing Myc, one of the hits was *PRKAA1*, encoding AMPK-α1 (Liu et al., 2012). Taken together, these results suggest that while AMPK-α2 might indeed be a tumor suppressor, AMPK-α1 might be an oncoprotein that promotes the survival of tumor cells, possibly by protecting them under conditions of energy stress. Consistent with this, recent analyses of the human cancer genome databases

revealed that while the *PRKAA2* gene (encoding α2) is quite often mutated in human cancers, *PRKAA1* tends to be amplified instead (Monteverde et al., 2015; Ross et al., 2016).

Vila et al (2017) were interested in the role of UBE2O, a large ubiquitin ligase with both E2 and E3 activities. *UBE2O* is located within a chromosomal region (17q25) that is amplified in some human cancers. They first generated *Ube2o* knockout mice, and found that MEFs derived from these grew more slowly and were more resistant to transformation in vitro than wild type controls, while UBE2O over-expression had the opposite effects. By crossing *Ube2O* knockout mice with two genetically engineered mouse models of breast and prostate cancer, they found that the severities of the lesions were greatly reduced in the homozygous knockouts, with intermediate effects in heterozygotes.

To pinpoint downstream targets of UBE2O responsible for these effects, they identified interacting proteins by mass spectrometry, among which AMPK-α2 was particularly prominent. In cell-free assays, UBE2O could trigger K48-linked polyubiquitylation of AMPK-α2 in the presence of an E1. Of three previously predicted ubiquitylation sites on α2, only mutation of Lys470 in the C-terminal domain abolished the modification. Interestingly, Lys470 is conserved in α2 isoforms from other vertebrates, but is replaced by arginine in α1 isoforms. Consistent with the idea that K48-linked polyubiquitylation of α2 triggers its degradation, RNAi knockdown of UBE2O in HCT116 human colon carcinoma cells increased the stability of α2 but not α1, and also increased the phosphorylation of two AMPK targets, acetyl-CoA carboxylase (ACC) and Raptor, the latter a key component of the mTORC1 complex. Knockdown of α2, but not α1, reversed growth inhibition induced by UBE2O knockdown in HCT116 cells grown as mouse xenografts, while knockout of α2 but not α1 by CRISPR/Cas9 in a human haploid cancer cell line (HAP1) reversed both inhibition of growth and increased phosphorylation of AMPK targets induced by UBE2O knockdown. The authors also provided evidence that UBE2O depletion reprograms cells away from glycolysis towards oxidative metabolism, and that this was dependent on AMPK-α2. To demonstrate the applicability of the results to humans, they found an inverse correlation between expression of UBE2O and AMPK-α2, but not –α1, in breast cancer biopsies by immunohistochemistry. Finally, UBE2O has previously been shown to be potently inhibited by arsenite, which is currently undergoing clinical trials in several cancers. Supporting the idea that arsenite might be effective in part via inhibition of UBE2O, treatment of their breast and prostate

cancer mouse models with arsenite in vivo reduced the severity of the lesions to a similar extent as UBE2O deletion.

The results of Vila et al (2017) therefore fully support the idea that AMPK-α2 is a tumor suppressor. They not only identify the first downstream target for UBE2O, but also the first ubiquitin ligase that seems to specifically target AMPK-α2 for degradation. Interestingly, UBE2O (originally known as E2-230) is expressed particularly highly in reticulocytes, and was proposed to have a role in degradation of proteins that are absent from the erythrocytes that develop from them (Wefes et al., 1995), one of which is AMPK-α2 (Foretz et al., 2011). Aberrant amplification of chromosomal region 17q25 may, however, lead to over-expression of UBE2O in cancer cells derived from other cell types, causing increased degradation of AMPK-α2 that might then promote the more glycolytic metabolism typical of tumor cells, while reducing expression of the tumor suppressor p53.

The idea that AMPK-α2 is a tumor suppressor, while AMPK-α1 is a tumor promoter, is superficially attractive, but it is already clear that this is an over-simplification. Pineda et al (2015) reported that the melanoma antigens MAGE-A3/-A6 recruit the ubiquitin ligase TRIM28 to AMPK-α1 and triggers its degradation. MAGE-A3/-A6 are closely related tumour antigens that are normally only expressed in testis, but are aberrantly re-expressed in some tumors. By triggering polyubiquitylation and proteasomal degradation of AMPK-α1, MAGE-A3/-A6 appear to promote tumor formation. This story has obvious echoes of the role of UBE2O in reticulocytes and in cancer, except that the AMPK isoform affected is α1 rather than α2. Clearly, it remains a challenge to fully understand the context-dependent roles of α1 and –α2 in different cancers.

Grahame Hardie is a Wellcome Trust Investigator and is also supported by Cancer Research UK.

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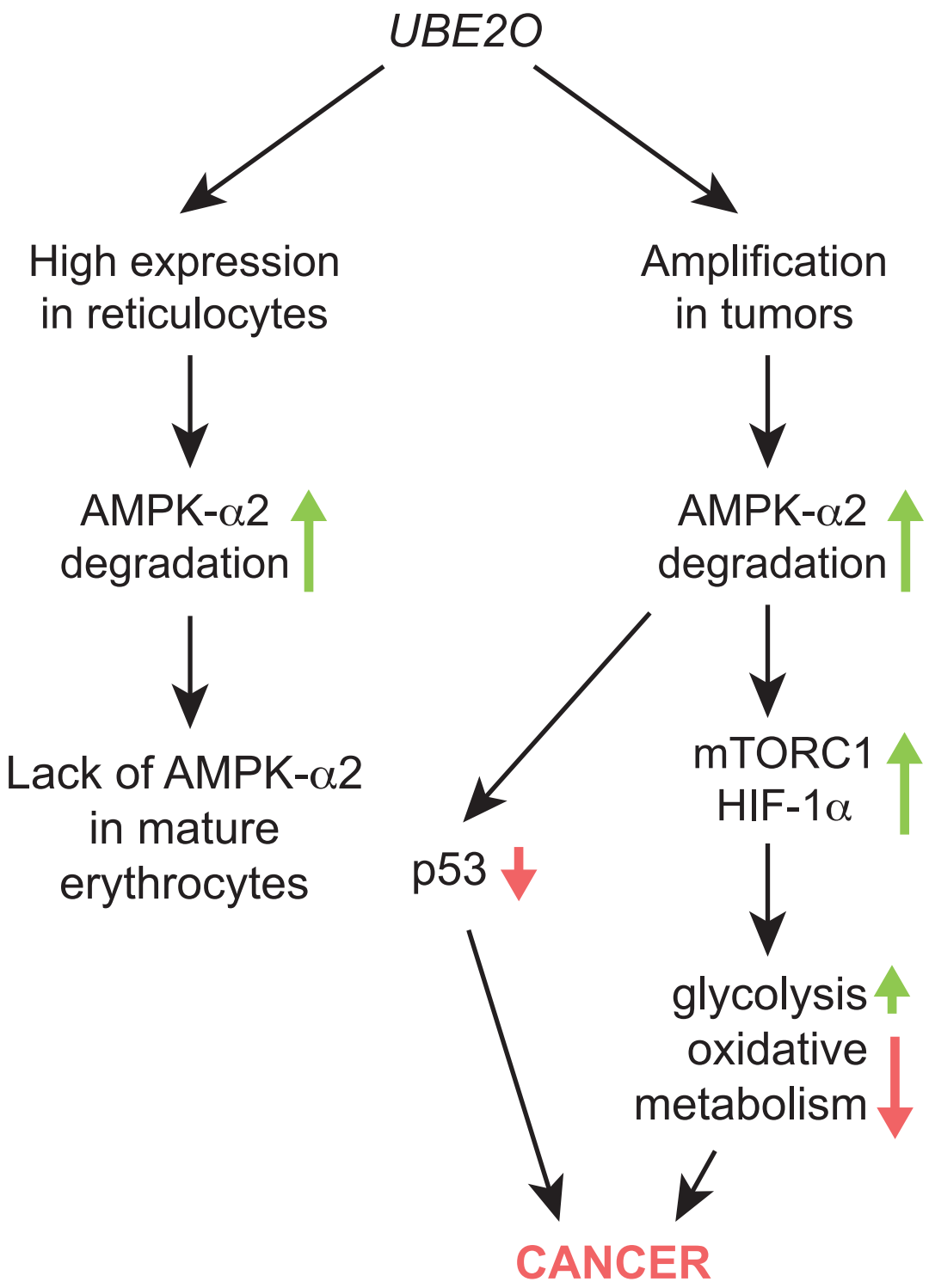
Figure: **Normal role of UBE2O in erythrocytes, and aberrant role in cancer.** UBE2O is highly expressed in reticulocytes, where its role may be to remove proteins not found in mature erythrocytes, including AMPK-α2. However, amplification of *UBE2O* in some tumors also leads to degradation of AMPK-α2, conferiing growth advantages on the tumor cells.


UBE2O
High expression in reticulocytes
Amplification in tumors
AMPK-α2 degradation
AMPK-α2 degradation
Lack of AMPK-α2 in mature erythrocytes
p53
mTORC1 HIF-1α
glycolysis
oxidative metabolism
CANCER